BIOSPHERE 2 REEXAMINED: SPECIES COMPOSITION AND INTERACTIONS WITHIN A HUMAN-CONSTRUCTED ECOSYSTEM

by

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Abstract

All ecosystems on the planet today experience some sort of human impact, causing changes in network composition, species diversity, the presence of invasive speices. Understanding how species composition changes in human-disturbed ecosystems has never been more important, and can be studied within research facilities such as Biosphere 2, a structure with several biomes modeled on different ecosystem. This study examines the current arthropod species composition of Biosphere 2 through species surveys, and investigates a mutualism between ants and a mealybug (Hemiptera: Pesudococcidae), in the rainforest biome of Biosphere 2. The species surveys asked how species composition has changed since past surveys and how the species composition of Biosphere 2 compares to the surrounding desert area. Then, to determine the mutualism's effects on mealybug numbers, four treatments were conducted: A+P+ (where ants and putative predators such as spiders were allowed access to mealybug populations), A-P+ (where ants were excluded but predators were allowed), A+P- (where ants were allowed but predators were excluded), and A-P- (where both ants and predators were excluded). I found species richness to have decreased within Biosphere 2 compared to past recorded invertebrate taxa numbers, as well as high abundances of *Paratrechina longicornis*, an invasive ant found in past surveys, within Biosphere 2. All genera of ants found within Biosphere 2 were also found in the surrounding desert area, suggesting Biosphere 2 is having an impact on the surrounding native ecosystem. To determine if a mutualism is occurring, and linear mixed models were run to compare differences in mealybug numbers from specific treatments to each other. I conclude the evidence for a mutualism is inconsistent and if one is occurring, the effect is extremely weak. In the future, species surveys and arthropod research within Biosphere 2 should be continued, to aid in further understanding of species composition and interactions in human-disturbed ecosystems.

Chapter 1: Biosphere 2 Reexamined: Species Composition Within a Human-Constructed Ecosystem

Introduction

“Pristine” ecosystems, as traditionally studied in ecology, are a concept of a bygone era; in the present age there are no places left on Earth that humans do not affect in some way (Gallagher & Carpenter 1997, Western 2001). Human-impacted ecosystems experience significant changes in species diversity, network composition, and the presence of invasive species, which often depend on disturbance to maintain their populations (Western 2001, Luken 1997). Additionally, human-facilitated climate change is causing a global redistribution of species at varying rates, resulting in novel biotic communities through the disruption of key species interactions and the emergence of entirely new interactions (Pecl et al. 2017). As human influences continue, predicting how species composition changes in semi-natural ecosystems has never been more important. Biosphere 2, a unique 180,000 m^3 structure located in Oracle, Arizona, offers an opportunity to study composition change not only within a semi-natural ecosystem, but within one fully constructed by humans (Nelson et al. 1993). Three sets of surveys, focusing on ants and other arthropods, were carried out within Biosphere 2 between 1990 and 1997, but these communities have not been examined since. I conducted five species surveys documenting arthropods in Biosphere 2 in 2018. Results from surveys reported here will contribute to our understanding of how species composition changes within human-constructed ecosystems, as well as how human-constructed ecosystems affect and are affected by their surrounding environment.

Biosphere 2 is composed of five individual ecosystems, which the designers termed “wilderness biomes”: a rainforest, savanna, desert, ocean, and mangrove marsh. In addition, there was an “anthropogenic biome wing”, originally comprised of a human habitat and agricultural area (Nelson et al. 1993). Biosphere 2 was initially built to conduct closed-system experiments. The whole structure was sealed off from the outside with a team of people inside, to test the ability of such ecological systems to support human life. These experiments were seen as a way to collect data on closed life-support systems and to determine their viability as long-term habitation systems on “space bases” (Nelson et al. 1993). Two experiments were carried out during the 1990s: Mission One, for two years with a team of eight, and Mission Two, for six months with a team of seven.

The goal of this research was to determine which arthropod species are found in Biosphere 2, how this has changed over time, and their diversity compared to the surrounding desert ecosystem. Species surveys carried out before the first closure identified about 3000 animal and plant species in the structure. Presently, Biosphere 2 staff maintains and monitors the terrestrial plants in each biome (Nelson et al. 1993). Therefore, the plant community has changed over time as it might in nature. Although several vertebrate species, including skinks, birds, lizards, and even *Garnetii garnetii*, an African galago, were introduced to Biosphere 2 before its initial closure, vertebrates were largely reported to have gone extinct by the end of the closure experiments (Nelson et al. 1993, Cohen & Tilman 1996).

Arthropods, and particularly ants, have had a relatively well-documented presence within the facility that has changed throughout time. After construction of Biosphere 2 and before the first closure, approximately 150 insect species were reported, including several dozen raised within the Biosphere 2 insectary (Nelson et al. 1993). Insects introduced intentionally were chosen to fulfill different functions within communities and with redundancy within food webs in mind, with the hope of offsetting eventual species extinctions (Nelson et al. 1993). For instance, eleven ant species were intentionally introduced to fulfill functions such as the recycling of dead animal and plant matter, and seed dispersal (Wetterer et al. 1999). However, arthropod introductions, as a whole, were less closely regulated than those of plants and vertebrates, and many local Arizona species entered and remained in the structure during construction (Wetterer et al. 1999) or were intentionally introduced before closure (J.L. Bronstein, pers. comm.). Insects were also accidentally introduced to the system via building material, plant samples, and soil samples. This included, for example, the Australian cockroach, *Periplaneta australasiae*, which entered Biosphere 2 on tropical plants installed in the rainforest (Silverstone & Nelson 1996). Plant pests such broad mites, pill bugs, root knot nematodes, aphids, thrips, and cockroaches were also observed before and during the first closure experiment (Silverstone & Nelson 1996). Several predatory insects, including a species of ladybird, *Cryptoaemus montouzieri*, and a parasitic wasp, *Aprostocetus hagenowii*, were introduced before the initial closure experiment to control the aforementioned pests, although they had mixed results (Silverstone & Nelson 1996).

Three periods of arthropod surveys were carried out in the 1990s: one in 1990-91 (before the first closure experiment), one in 1993-94 (during the first closure experiment and after it ended), and one in 1996-97 (Wetterer et al. 1999). Data from these surveys were collected and published in one paper. Data included ant species present in each of the three survey periods, and the number and species of other arthropods in the facility (in the 1996-97 survey) (Wetterer et al. 1999). Information on arthropods other than ants found in the two earlier surveys was not published. It is therefore unclear exactly what arthropods were present at different points in Biosphere 2 before the 1996-97 surveys. Cockroaches and katydids were reported to be abundant after the first closure experiment, but their exact numbers were unfortunately not recorded (Cohen & Tilman 1996). However, the species of ants, as well as their status as invasive or native, were well-recorded in each survey. By the second round of surveys, in 1993-94, researchers reported that numbers of an invasive ant, *Paratrechina longicornis* were rising, whereas intentionally introduced insects had died out (Marino & Odum 1999, Cohen & Tilman 1996, Wetterer et al. 1999). *Paratrechina longicornis* was not purposely introduced to Biosphere 2. It is considered an invasive tramp ant: a species associated with human disturbance and often spread through commerce (Wetterer 2008). Its geographic origin is unknown, though it is hypothesized to be native to Southeast Asia and Melanesia (Wetterer 2008). The presence of invasives throughout Biosphere 2 is not unexpected, as human-impacted systems have an established track record of invasive species rising to abundance (King & Tschinkel 2008, Orians 1986).

In the twenty-two years since the surveys reported in Wetterer et al. (1999), no arthropod surveys have been conducted in Biosphere 2. Neither the species composition nor how it has changed over the past two decades is known. Understanding how species composition has changed over time will lend insight into the ecology of human-constructed ecosystems, structures that may become more common as numbers of humans and their impact on Earth grows.

To address these topics, I conducted five species surveys over the course of 2018, both within the wilderness biomes and in the outside desert area surrounding Biosphere 2. The aims were to determine (a) which arthropod species are currently present in Biosphere 2, (b) whether *P. longicornis* is still present at high numbers relative to other arthropods, and (c) how species composition of the surrounding desert outside Biosphere 2 compares to the species composition inside. This last question sheds insight into the fluidity of boundaries between human-constructed ecosystems and natural ones. It is not known if native species are colonizing the biomes within Biosphere 2, nor whether non-native species within Biosphere 2, are escaping and establishing within the relatively undisturbed Sonoran Desert habitat surrounding the structure. Comparing species composition within the biomes of Biosphere 2 and the surrounding desert area speaks to this issue.

Methods

Species Surveys

Biosphere 2 was designed to mimic the variation seen in Earth's different biomes. The original "wilderness biomes" are located in the eastern wing of Biosphere 2, an area 165 m x 30-44 m (Nelson et al. 1993). There are five biomes: desert, savanna, ocean, marsh, and rainforest (Nelson et al. 1993). They are differentiated by plant species composition, as well as by distinct abiotic factors, including rainfall, soil and sediment type, water quality, wind flow, temperature, pH, light, and humidity (Allen and Nelson, 1999). They were designed to represent different ecosystem types, rather than to mimic specific locations on Earth. For instance, it is known that the savannah biome contained species from Australia, Africa, Florida, and South America. Importantly, however, there appear to have been no published species lists (Nelson et al. 1993). Biosphere 2 also contains an orchard that was originally part of the Intensive Agricultural Biome (IAB), which provided food for the original inhabitants (Marino et al. 2002). I included the orchard area in the species surveys, along with the rainforest, savanna, and desert biomes. I did not survey the ocean and marsh biomes. I also included the outside desert area immediately surrounding Biosphere 2. The surrounding habitat is Sonoran Desert, with common plant species that include *Larrea tridentata* and annual grasses such as *Bouteloua aristidoies* (Avise 1994, Niering and Lowe 1984). This project was not designed to exactly replicate the survey methods of Wetterer et al. 1999. Methods used in the earlier surveys were incompletely described and varied among the three survey periods, so this would not have been possible. However, all survey results are included in the same tables below, to permit comparisons of species composition.

I conducted five arthropod species surveys throughout 2018. The surveys lasted for two days each, in January, March, June, August, and October. In each survey, 10 pitfall traps, 10 tuna baits, and 10 honey baits were placed in the rainforest, savanna, and desert biomes, as well as in the orchard and outside areas. After placing pitfall traps, I secured the tuna and honey baits at a on the same branch of whichever plant was nearest to the pitfall traps with zip ties. This ensured that the pitfall traps were sampling the leaf litter, while the tuna and honey baits were sampling potential arboreal species. The pitfall traps were made from 113.40 g, 7.94 x 5.24 cm plastic containers filled 5.05 g full of soapy water, dug into the ground with their rims at the soil surface level. The soap in the water ensured surface tension sufficient to prevent any arthropods that fell into the traps from staying afloat and escaping. The bait traps were 1.5 mL plastic vials filled with 1.125 mL of either canned tuna or honey. I placed the pitfall traps haphazardly throughout each biome and the orchard. For the outside survey, I placed the traps haphazardly around the perimeter of Biosphere 2, anywhere from 10-20 m from the walls of the structure, avoiding concrete walkways. Following the same procedure as the inside surveys, I secured the tuna and honey baits on the same branch of which plant was nearest to the pitfall traps with zip ties. I collected all traps and baits 48 h after placing them, and euthanized any living arthropods in the baits by placing the baits in a freezer. All specimens in the pitfall traps were no longer alive due to the water in the traps.

Additionally, one blacklight trap was set out in the rainforest biome in August for three hours from 6-9 pm and again in the desert biome in October for three hours from 6-9 pm. Blacklight traps were not set in all the biomes as there are limited flying taxa within Biosphere 2 and few species were attracted to the light. Species found from blacklight traps in the rainforest biome are recorded in Table 12, but species found from blacklight traps in the desert biome were not recorded.

Arthropod Identification

After the traps were collected, I examined all specimens found in the pitfall traps, tuna baits, and honey baits under a dissecting microscope within one week of collection, to ensure that the specimens remained identifiable. I identified *Paratrechina longicornis* to species, all other ants to genus, and any other arthropods to order. Ants were identified using Fisher and

Cover (2007). I also recorded the number of each taxon found in each trap or bait. All specimens were preserved in ethanol and will be deposited in the University of Arizona Insect Collection. I followed this procedure for all five surveys.

Results

Table 1 shows all invertebrates found in soil and leaf litter surveys and baits, in surveys conducted from 1996-1997, as published in Wetterer et al. 1999, as well as arthropods found in pitfall traps, tuna baits, and honey baits, in the five surveys I conducted in 2018. In 1996-97, 16 distinguishable taxa of arthropods were found, but this fell to six in 2018. However, Wetterer et al. 1999 reported laying out 18 soil and leaf litter surveys throughout June 1997. I did not include soil and leaf litter surveys in my sampling, making it much less likely for me to find some of the taxa found in Wetterer et al. 1999. It was also reported that 28,827 *P. longicornis* were collected at 174 bait stations in the final 1996-97 surveys, however this number only includes *P. longicornis* found in baits, and not in other survey methods carried out in this survey period (Wetterer et al. 1999). Therefore, in Table 1, I recorded this number as 28,000+ to include ants found through observation and soil and leaf litter surveys. The numbers for Hymenoptera I recorded in my surveys are reported on in more depth in Tables 3, 4, and 5.

Table 1: Invertebrates Found in Surveys Carried Out Inside Biosphere 2

Invertebrates	Surveys 1996-1997	Survey Jan-2018	Survey Mar-2018	Survey Jun-2018	Survey Aug-2018	Survey Oct-2018
Mites	1271					
Isopoda	323	2		1	33	
Millipedes	74					
Springtails	39					
Earthworms	12					
Hemiptera	8					
Araneae	7	2		3	3	7
Webspinners (Embioptera)	7					
Snails (Gastropoda)	5					
Termites (Isoptera)	4					
Cockroaches (Blattodea)	3	59	2	11	17	4
Beetles (Coleoptera)	3					
Wasps (Hymenoptera)	2					
Caterpillar (Lepidoptera)	1					
Lacewing (Neuroptera)	1					
Diplura	1					
Diptera		3	3		1	35
Orthoptera		67	3	15	91	75
(Ants) Hymenoptera	28,000+	22,141	2933	15,334	5918	1902

Table 1: Presence and number of invertebrates found in Biosphere 2 from 1997-2018. Surveys 1996-1997 is comprised of data from Wetterer et al. 1999, and were collected through soil and leaf litter surveys and baits. The notation for the other surveys listed indicates the month and year they were done. The 2018 surveys include data collected from pitfall traps, tuna baits, and honey baits.

Table 2 shows all arthropod taxa found in the surrounding desert area outside Biosphere 2, from the surveys I conducted in January, March, June, August, and October of 2018. The majority of arthropods found were Hymenoptera, usually ants, but parasitoid wasps were also found. Over the course of all five surveys, several individual Diptera, individuals from the order Araneae, one individual Coleopteran, and one individual Orthopteran was found.

Table 2: Arthropods Found in Surveys Carried Out Outside Biosphere 2

Arthropods	Survey Jan-2018	Survey Mar-2018	Survey Jun-2018	Survey Aug-2018	Survey Oct-2018
Araneae	1			1	
Coleoptera	1				
Hymenoptera	67	68	699	335	160
Diptera	1	7	5		
Orthoptera				1	

Table 2: Presence and number of arthropods, found outside Biosphere 2 in 2018. The notation for the surveys indicates the month and year they were done. Data included was collected from pitfall traps, tuna baits, and honey baits.

Table 3 lists species of ants found in each survey carried out inside Biosphere 2 (reported as a group in Table 1 under the order Hymenoptera). The table shows species found in past surveys reported in Wetterer et al. 1999, as well as genera I found in 2018. Only the ant genera *Paratrechina, Monomorium, Phidole*, and *Odontomachus* were found inside Biosphere 2 throughout the 2018 surveys. In the final survey recorded in Wetterer et al. 1999, carried out from 1996-1997, ten genera of ants had been found. Two decades later in 2018, the maximum number of genera of ants found in a single survey was three.

Table 3: Ant Species Found in Surveys Carried Out Inside Biosphere 2

Ant Species	Status	Surveys 1990-91	Surveys 1993-94	Survyes 1996-97	Survey Jan-2018	Survey Mar-2018	Survey Jun-2018	Survey Aug-2018	Survey Oct-2018
Odontomachus clarus	N		x						
Crematogaster opuntiae	N	x							
Linepithema humile	T	x							
Paratrechina bourbonica	T	x							
Pheidole sp. 2	?	x			x				
Pheidole hyatti	N	x	x						
Solenopsis xyloni	N	x							
Cardiocondyla ectopia	T	x	x						
Hypoponera apaciceps	T	x	x	x					
Paratrechina longicornis	T		x	xxxx	xxxx	xxx	xxxx	xxx	xxx
Tetramorium bicarinatum	T		x	x					
Solenopsis molesta	N			x					
Forelius mccooki	N			x					
Cardiocondyla wroughtoni	T			x					
Monomorium floricola	T			x					
Tapinoma melanocephalum	T			x					
Paratrechina vividula	T			x					
Strumigenys rogeri	T			x					
Odontomachus sp.	?						x		
Monomorium sp.	?				x	x			

Table 3: Presence of ants in Biosphere 2 from 1990-2018. Each "x" denotes the presence of a species, with "xxx" signifying a number of individuals above 1000, and "xxxx" signifying a number of individuals above 10,000. This table includes data from surveys I conducted and data from surveys reported in Wetterer et al. 1999. 2018 survey data includes species found in pitfall traps, tuna baits, and honey baits. This table also includes all indoor biome traps included in sampling: the rainforest, desert, savanna, as well as the orchard. Species are listed as "N" for "native" when they are considered to be native to the region of Arizona Biosphere 2 is located in, or "T" for "tramp" when they are considered to be invasive to the region of Arizona where Biosphere 2 is located. If their status as native or invasive is unknown, their status is indicated as so with a question mark.

Tables 4 and 5 show the proportion of ants found in pitfall traps, and tuna and honey baits within Biosphere 2. The proportions of ants found in past surveys were not included in this table, as they were not published. As Tables 4 and 5 show, *Paratrechina longicornis* were found most often in both pitfall traps and baits, compared to other ants found. Every other genus of ant found was only seen in either one out of the 40 pitfall traps set inside Biosphere 2 (as their presence was a proportionality of 0.025), or one out of the 80 baits set inside Biosphere 2 (with a proportionality of 0.0125). *Paratrechina longicornis* was the only ant found in pitfall traps, and all baits, in all five surveys.

Table 4: Proportions of Ants Found in Pitfall Traps Inside Biosphere 2

Ant Species	Status	Survey Jan-2018	Survey Mar-2018	Survey Jun-2018	Survey Aug-2018	Survey Oct-2018
Monomorium sp.	?		0.025			
Odontomachus sp.	?			0.025		
Paratrechina longicornis	T	0.9	0.925	0.95	0.825	0.875
Pheidole sp.	?	0.025				

Table 4: Proportions of ants found in pitfall traps inside Biosphere 2. Proportions were calculated as the number of pitfall traps a species of ant was found in, over all 40 pitfall traps from the surveyed biomes. Species status is indicated as "T" for tramp, if they are known to be invasive in the desert area around Biosphere 2, or "?" if their status is unknown.

Table 5: Proportions of Ants Found in Tuna/Honey Baits Inside Biosphere 2

Ant Species	Status	Survey Jan-2018	Survey Mar-2018	Survey Jun-2018	Survey Aug-2018	Survey Oct-2018
Monomorium sp.	?	0.0125	0.0125			
Odontomachus sp.	?					
Paratrechina longicornis	T	0.588	0.425	0.475	0.425	0.4625
Pheidole sp.	?					

Table 5: Proportions of ants found in tuna and honey baits inside Biosphere 2. Proportions were calculated as the number of tuna and honey baits a species of ant was found in, over all 80 baits from the surveyed biomes. Species status is indicated as "T" for tramp, if they are known to be invasive in the desert area around Biosphere 2, or "?" if their status is unknown.

Tables 6 and 7 show the proportion of ants found in pitfall traps, and tuna and honey baits, outside Biosphere 2. Over the course of the 2018 surveys, I found ten genera of ants in the desert area outside Biosphere 2. All four genera of ants found in Biosphere 2 in the most recent surveys were also found in the surrounding desert area. *Paratrechina* was identified to species as *P. longicornis*. As Tables 6 and 7 show, *P. longicornis* was found most often in both pitfall traps and baits. They were found in all pitfall traps in all five surveys, and in baits in four out of the five surveys, making them the only ant found outside in all five surveys.

Table 6: Proportions of Ants Found in Pitfall Traps Outside Biosphere 2

Ant Species	Status	Seen in B2?	Survey Jan-2018	Survey Mar-2018	Survey Jun-2018	Survey Aug-2018	Survey Oct-2018
Crematogaster sp.	?				0.7	0.2	0.1
Formica sp.	?		0.1				
Monomorium sp.	?	Yes			0.2		
Odontomachus sp	?	Yes			0.5	0.5	0.2
Paratrechina longicornis	T	Yes	0.2	0.3	0.8	0.3	0.3
Pheidole sp.	?	Yes		0.2	0.4	0.3	
Solenopsis sp.	?			0.1		0.3	
Tapinoma sp.	?						
Technomyrmex sp.	?			0.2			
Tetramorium sp.	?						

Table 6: Proportions of ants found in pitfall traps outside Biosphere 2. Proportions were calculated as the number of pitfall traps a species of ant was found in, over all ten pitfall traps placed outside. Species status is indicated as "T" for tramp, if they are known to be invasive in the desert area around Biosphere 2, or "?" if their status is unknown.

Table 7: Proportions of Ants Found in Tuna/Honey Baits Outside Biosphere 2

Ant Species	Status	Seen in B2?	Survey Jan-2018	Survey Mar-2018	Survey Jun-2018	Survey Aug-2018	Survey Oct-2018
Crematogaster sp.	?						
Formica sp.	?						
Monomorium sp.	?	Yes				0.05	
Odontomachus sp	?	Yes					
Paratrechina longicornis	T	Yes		0.05	0.4	0.05	0.05
Pheidole sp.	?	Yes	0.1				0.05
Solenopsis sp.	?						
Tapinoma sp.	?			0.05			0.35
Technomyrmex sp.	?						
Tetramorium sp.	?						0.05

Table 7: Proportions of ants found in tuna and honey baits outside Biosphere 2. Proportions were calculated as the number of tuna and honey baits a species of ant was found in, over all 20 baits placed outside. Species status is indicated as "T" for tramp, if they are known to be invasive in the desert area around Biosphere 2, or "?" if their status is unknown.

Tables 8-11 show the species composition of each biome as seen in each of the five 2018 surveys. *Paratrechina longicornis* and Blattodea were seen in all the biomes. However, Orthoptera were mainly found in biomes with a drier climate (the savanna and the desert). The species richness for ant genera was highest within the desert biome, where three genera of ants were found.

Table 8: Rainforest Biome Arthropod Totals

Order/Species	Survey Jan-2018	Survey Mar-2018	Survey Jun-2018	Survey Aug-2018	Survey Oct-2018
Araneae	1		2	3	1
Blattodea	30	1	5	2	
Diptera					1
Isopoda	2		1	33	
Orthoptera			1		
Paratrechina longicornis	2774	187	1406	663	102

Table 8: Orders and species found within the rainforest biome during surveys carried out inside Biosphere 2 in 2018. Data included was collected from pitfall traps, tuna baits, and honey baits.

Table 9: Orchard Biome Arthropod Totals

Order/Species	Survey Jan-2018	Survey Mar-2018	Survey Jun-2018	Survey Aug-2018	Survey Oct-2018
Araneae	1				
Blattodea	11		7		2
Diptera	2		1		31
Isopoda			2		
Orthoptera				1	
Paratrechina longicornis	1525	125	898	817	377
Pheidole sp.	1				

Table 9: Orders and species found within the orchard biome during surveys carried out inside Biosphere 2 in 2018. Data included was collected from pitfall traps, tuna baits, and honey baits.

Table 10: Savanna Biome Arthropod Totals

Order/Species	Survey Jan-2018	Survey Mar-2018	Survey Jun-2018	Survey Aug-2018	Survey Oct-2018
Araneae					6
Blattodea	2	1		4	1
Diptera	1	3			3
Monomorium sp.		9			
Orthoptera	2			34	10
Paratrechina longicornis	11,151	738	9540	3434	237

Table 10: Orders and species found within the savanna biome during surveys carried out inside Biosphere 2 in 2018. Data included was collected from pitfall traps, tuna baits, and honey baits.

Table 11: Desert Biome Arthropod Totals

Order/Species	Survey Jan-2018	Survey Mar-2018	Survey Jun-2018	Survey Aug-2018	Survey Oct-2018
Araneae			1		
Blattodea	14			11	1
Diptera				1	
Monomorium sp.	56				
Odontomachus sp.			4		
Orthoptera	65	28	14	56	65
Paratrechina longicornis	6181	1874	3231	1004	1165

Table 11: Orders and species found within the desert biome during surveys carried out inside Biosphere 2 in 2018. Data included was collected from pitfall traps, tuna baits, and honey baits.

Table 12 shows species caught in a blacklight trap in the rainforest biome of Biosphere 2. The trap caught one Orthopteran and six flying *P. longicornis*. The *P. longicornis* were thought to be queens or males, as both have wings.

Table 12: Arthropods Found in Blacklight Traps in Rainforest Biome

Order/Species	Blacklight Trap Aug-2018
Paratrechina longicornis	6
Orthoptera	1

Table 12: Orders and species found within the rainforest biome when a blacklight trap was set up over the course of three hours from 6-9pm

Tables 13 and 14 show the proportion of ants found in tuna baits and the proportion of ants found in honey baits. *Paratrechina longicornis* were found at higher proportions in honey baits than in tuna baits. Additionally, *Monormorium* was found in honey baits in two surveys, but not seen in tuna baits.

Table 13: Proportions of Ants Found in Tuna Baits Inside Biosphere 2

Ant Species	Survey Jan-2018	Survey Mar-2018	Survey Jun-2018	Survey Aug-2018	Survey Oct-2018
Paratrechina longicornis	0.425	0.4	0.425	0.425	0.35

Table 13: Proportions of ants found in tuna baits inside Biosphere 2. Proportions were calculated as the number of tuna baits a species of ant was found in, over all 40 baits from the surveyed biomes.

Table 14: Proportions of Ants Found in Honey Baits Inside Biosphere 2

Ant Species	Survey Jan-2018	Survey Mar-2018	Survey Jun-2018	Survey Aug-2018	Survey Oct-2018
Paratrechina longicornis	0.75	0.625	0.55	0.475	0.6
Monomorium sp.	0.025	0.025			

Table 14: Proportions of ants found in honey baits inside Biosphere 2. Proportions were calculated as the number of honey baits a species of ant was found in, over all 40 baits from the surveyed biomes.

Discussion

Species diversity within Biosphere 2 has changed since surveys ended in 1997. Species richness among arthropods has decreased overall, compared to past surveys. I found four genera of ants present within Biosphere 2 and six orders of arthropods, while at the time of the last surveys from 1996-97, nine genera of ants and sixteen orders of arthropods were found. Importantly however, I did not include soil and leaf litter samples in my survey methods. Therefore, I was unlikely to find certain taxa such as mites, isopods, snails, millipedes, and earthworms as these organisms are much less likely to fall into pitfall traps. This likely accounts for some of the difference in species richness seen between the 1996-97 survey period and the 2018 surveys. Definitive changes in species abundance are also difficult to determine. Methods for past surveys were not given in detail in the previous paper, and were not standardized across surveys, but instead, the number and types of survey methods varied for each survey period. The 1990-91 and 1993-94 survey periods only recorded species richness for ants. The 1996-97 survey period recorded species richness for all ants and species abundance specifically for *P. longicornis*, as well as species richness and abundance for other arthropods. In the 1996-97 surveys, observers determined arthropod (excluding ant) numbers from one round of collecting 18 l-liter soil and leaf litter samples (Wetterer et al. 1999), whereas I determined current arthropod numbers from 40 pitfall traps, 40 tuna baits, and 40 honey baits, inside of Biosphere 2, repeated a total of five times. Compared to the 1996-97 survey period, I increased the number and frequency of traps and baits for arthropod sampling in 2018. Therefore, it could be that orders such as Araneae and Isopoda, have decreased in abundance over time, as I found fewer individuals of those orders with increased sampling methods. It is also likely that I would have recorded Isopoda, at higher abundances, if I had done soil and leaf litter sampling, or other methods aimed at capturing less mobile organisms. It is possible though, to conclude that Orthoptera and Diptera have increased in abundance within Biosphere 2, as they were entirely absent in the 1996-97 survey period. However, for orders such as Blattodea, it is more difficult to draw conclusions, as recording more individuals could be a result of increased sampling methods in 2018. The results suggest the fourteen orders found in past surveys, that I did not find within Biosphere 2, decreased in abundance, though it is likely some are still present and soil and leaf litter surveys would have found them.

Although it is not possible to rigorously compare my data to those from past surveys due to different sampling techniques, numbers of *P. longicornis* have clearly increased since the 1996-97 surveys. It was reported for the 1996-97 survey period that 28,826 *P. longicornis* were found over a 1-month period, from 174 baits stations set out at five-meter intervals throughout the wilderness areas (Wetterer et al. 1999). I found 22,084 *P. longicornis* during Survey Jan-2018, over a 48-hour period from 40 pitfall traps and 80 baits (Table 3). This means that around three-quarters of the *P. longicornis* found over a 1-month timeframe in the 1996-97 survey period, were found in two days in 2018, suggesting that numbers of *P. longicornis* have increased. *Paratrechina longicornis* is currently the most abundant ant present in Biosphere 2. Of all the ants I found in Biosphere 2, 99.85% were *P. longicornis*. *Paratrechina longicornis* were also found at higher proportions in honey baits than they were in tuna baits, though they were still seen in baits regardless of type for every survey inside Biosphere 2 (Tables 13 and 14).

Paratrechina longicornis is a species whose natural history is uniquely suited for a human-impacted system such as Biosphere 2. It is considered an opportunist species, one that is unspecialized with about average aggression, that lives mainly in disturbed urban and coastal environments (Lester 2005, Wetterer 2008). *Paratrechina longicornis* has been hypothesized to be native to Southeast Asia (Wetterer 2008). It has been recorded in over 2100 sites around the globe, and likely became so widespread because it survives well in artificial environments such as ships at sea (Wetterer 2008). In the United States, *P. longicornis* has been recorded in 23 states, mainly in the southeast, though it has also been recorded in surveys in the southwest, including San Diego, Phoenix, and Tucson (Wetterer 2008, Field et al. 2007). A survey of southwestern ants for pest management purposes found a single *P. longicornis* outside a Phoenix residential area, and reported *P. longicornis* to make up 2% of the 113 individual ants recorded, on calls to Tucson residential areas (Field et al. 2007). In behavioral assays conducted with invasive ant species, *P. longicornis* showed indifferent or evasive behavior when confronted with other invasive ant species, though authors speculated *P. longicornis* could be more aggressive towards native species (Bertelsmeier et al. 2015). It is an omnivore, and has been known to feed on honeydew, seeds, fruit, dead or live insects, as well as on household food (Kenne et al. 2005). Its competitive interactions with other species depend on foragers' ability to quickly recruit workers when a food source is found, rather than to defend against or repel competitors (Kenne et al. 2005). Low ant diversity, and consequently low competition, within Biosphere 2 could therefore be one of the factors ensuring their abundance. *Paratrechina longicornis* have been shown to be displaced at baits and nests where more aggressive ants such as *Solenopsis invicta*, *Pheidole megacephala*, or *Wasmannia auropunctata* are present (Banks & Williams 1989, Way & Bolton 1997). However, more aggressive ants are either not present within the facility, or present at such low numbers that they may not affect *P. longicornis* population numbers.

Species composition of the desert area surrounding Biosphere 2 differs from the inside of the facility. I found five orders of arthropods in pitfall traps outside of the structure. The majority of arthropods found outside were which were ants. The desert had a higher diversity of ants, however, even though this area has more possible predators, more competition, and harsher weather conditions than inside the facility. Over the course of five surveys, I found ten ant genera outside, four of which I also found inside Biosphere 2. Notably, *P. longicornis* was found in every outdoor survey. Though *P. longicornis* has been found in outdoor surveys around Tucson residential areas, as Biosphere 2 is not located near residential areas, it is likely that the *P. longicornis* found in the desert area surrounding Biosphere 2 either originated within the facility, or are descended from ants who did (Field et al. 2007). Thus, the presence of *P. longicornis* in the outside desert has larger implications: its presence could indicate that it is establishing within the Sonoran Desert, at least in the vicinity of Biosphere 2. Taking into account how species successful in human-constructed ecosystems impact and infiltrate the native ecosystems is an important consideration to carry forward when considering the construction of human-built ecosystems. In particular, *P. longicornis* is adept at thriving within human-constructed ecosystems. It is often recorded within buildings and greenhouses. Examples include the Paris Botanical Garden greenhouses, the Orchid House of the Dorpat Botanical Gardens in Tartu, Estonia, and an airport building in Zurich, Switzerland (Wetterer 2008).

In summary, my surveys, when compared to past ones, show that species change is occurring within Biosphere 2, a human-constructed ecosystem. Species composition has apparently changed, and species richness has decreased, notably among the ants. *Paratrechina longicornis* is still present in Biosphere 2, and now exists at exceedingly high numbers relative to other ants. I also found this species in the desert surrounding Biosphere 2, which could suggest the facility is altering the species composition of the surrounding desert ecosystem. In the future, it is important to standardize surveying methods for systems like Biosphere 2, when species diversity should be consistently evaluated over time, and surveys will likely be done by different people. Arthropod surveys should be continued in Biosphere 2, as they will contribute additional insight to species composition within human-constructed ecosystems

Chapter 2: Interactions Between Invasive Ant *Paratrechina Longicornis* and Pseudococcidae Species in Biosphere 2

Introduction

A mutualism is an interaction between organisms that occurs when organisms have a reciprocally positive effect on the growth rate or fitness of their partners (Bronstein 2015). Protection mutualisms take place when one organism receives a food reward or protection against the physical environment, in exchange for protecting its partner from the negative impact of an abiotic or biotic environmental factor (Bronstein 2001). One such protection mutualism is common between ants and honeydew-producing Hemipteran insects. Ants feed on the carbohydrate-rich excretions (honeydew) Hemiptera produce, and in return protect the Hemiptera from predators and parasites (Way 1963, Hölldobler & Wilson 1990). This mutualism is well-documented between ants and different kinds of Hemiptera including mealybugs, aphids, and scale insects (Clark et al. 1982, Helms & Vinson 2002, Schuldt et al. 2017). However, these interactions are extremely context-dependent and can shift from mutualistic to commensal or antagonistic. This shift is dependent on factors such as the nutritional needs of the ants, the size of the Hemipteran populations, and the abundance of natural enemies (Ness & Bronstein 2004, Stadler & Dixon 2005). Ant-Hemipteran interactions are common in both natural and managed habitats, and are seen often in agricultural systems. Many studies have shown that plants indirectly benefit from ant-Hemipteran mutualisms, due to ants preying on herbivorous insects that are more harmful to the plants than Hemiptera (Styrsky & Eubanks 2007, Offenberg 2015, Schuldt et al. 2017). However, other studies show that the presence of ant-Hemipteran mutualisms result in damage to the host plant (Renault et al. 2005). Though outcome to the host plant can vary, ant-Hemipteran mutualisms have been determined to be so impactful to the surrounding arthropod and plant communities that they have been termed “keystone interactions” (Eubanks & Styrsky 2006). The prevalence of ant-Hemipteran interactions, and their impacts on the species around them, have economic significance for agricultural systems, making it important to further our understanding of how these interactions function in human-constructed environments.

Biosphere 2 is a human-constructed system comprised of different biomes modeled on Earth's ecosystems that is located north of Tucson, Arizona (Marino & Odum 1999). This structure is considered a highly-disturbed "natural" ecosystem (Wetterer et al. 1999). Biosphere 2 was originally constructed to carry out closed-system experiments. Today, it consists of five "model" systems: a rainforest, savanna, desert, mangroves, and an ocean, as well as an orchard, which is what remains of the original agricultural area (Nelson et al. 1993). It was originally reported that around 3000 species were put into Biosphere 2 before closure, though no official species lists were published (Nelson et al. 1993). Vertebrates were reported as extinct after the closure experiments concluded, but arthropod species persisted and were surveyed several times throughout the 1990s (Cohen & Tilman 1996, Wetterer et al. 1999). Arthropod species within Biosphere 2 have not, however, been surveyed since surveys were done from 1996-97, when it was reported that *Paratrechina longicornis* Latreille, commonly called the black crazy ant, was the most abundant species within the system (Wetterer et al. 1999). *Paratrechina longicornis* is an invasive species found worldwide. It has been known to participate in protection mutualisms with Hemiptera (Wetterer 2008, Díaz-Castelazo et al. 2010, Savage & Rudgers 2013). Wetterer et al. (1999) suggested that *P. longicornis* are able to exist at high numbers in Biosphere 2 because they are subsidized by the honeydew Hemiptera produce in Biosphere 2. Mealybugs were noted on different tree species, but it was not mentioned which biomes the mealybugs could be found in (Wetterer et al. 1999). Though the ant-tended mealybugs were considered "ant mutualists" at the time of past surveys, the effects *P. longicornis* had on mealybug numbers were not actually examined. Further, it was also not determined if putative predators of Hemiptera were present in Biosphere 2. The presence of putative mealybug predators is necessary for this interaction to be considered a mutualism, because without predators, the Hemiptera would not require protection and presumably would not benefit from this interaction.

Arthropod surveys that I conducted in 2018, reported in Chapter 1, found low arthropod species richness within Biosphere 2. *Paratrechina longicornis* was the most abundant species, though I also found spiders, a putative predator of the Hemiptera, to be present within the different biomes. A blacklight trap was set in the rainforest biome of Biosphere 2 in August 2018 to check for flying predators, but none were found. Spiders were thought to be preying on the

Hemiptera from observations, during pilot experiments. Ants were prevented from accessing a Hemipteran colony through applying Tanglefoot to the stem of a leaf and when the leaf was checked the following week, a spider was found on the leaf and the Hemipteran colony was gone. Though predation was not directly observed, and it is possible the Hemiptera in this colony died unrelated to the spider, their absence and the presence of a putative predator suggested the spider preyed on the Hemiptera. Additionally, *P. longicornis* were observed caught in spider's webs at different times throughout this study, so it is known spiders are preying on any available food source within Biosphere 2. The species identity of the Hemipteran has not been determined, but it is a mealybug in the family Pseudococcidae, and is referred to in the rest of this paper as "mealybugs". The mealybugs can be found throughout the rainforest biome of Biosphere 2, but was not observed in any of the other biomes at the times of these surveys.

In this study, I hypothesized that a protection mutualism was occurring between *P. longicornis* and honeydew-producing Hemiptera in the rainforest biome of Biosphere 2. To test this hypothesis, I conducted manipulative experiments denying and allowing different combinations of *P. longicornis* and putative predators access to the mealybugs. I then made predictions about how numbers of mealybugs should differ when pairs of treatments are compared (Table 1). First, comparing A+P+ to A+P-, there should be an increase in mealybugs in treatment A+P-. This comparison examines if predators are present in high enough abundance in the system to have an impact on mealybug numbers. If mealybug numbers do not increase when predators are prevented from accessing them, it suggests that the presence of predators is not having a significant effect on mealybug numbers. Second, comparing A-P+ to A-P-, there should be an increase in mealybugs in treatment A-P-. The comparison of these two treatments tests if there is a need for a mutualism in the system. If there are no ants present, and there is no significant difference in mealybug numbers when predators are present or not, it suggests that mealybugs do not require protection from ants, in which case, no protection mutualism is required. Third, comparing A+P+ to A-P+, the number of mealybugs in A-P+ should decrease. This comparison tests if ants are reducing the effects of predators. If there is no difference in mealybug numbers when ants are present versus when they are absent, it suggests that ants are not having an effect on mealybug predation. Finally, the comparison of A-P- to A+P- tests if ants themselves are having a negative effect on the mealybugs, e.g., consuming them. There should be no difference in mealybug numbers between these two treatments, as there is no threat of predation,

Table 1: Paired Treatment Comparison Predictions

Treatments Compared	Expected change in mealybugs
A+P+ v. A+P-	A+P- increase
A-P+ v. A-P-	A-P- increase
A+P+ v. A-P+	A-P+ decrease
A-P- v. A+P-	No change

Table 1: Predictions for changes in mealybugs numbers between two treatments.

Methods

I conducted all experiments in the rainforest biome of Biosphere 2, as I did not observe this species of mealybug in any of the other biomes at any point during this study. In the rainforest, I observed dense colonies on multiple leaves of mealybugs on *Clitoria racemosa*, *Coffea arabica*, and *Musa acuminata* trees. However, I only conducted experiments on *Clitoria racemosa* and *Coffea arabica*, as the *M. acuminata* leaf area and leaf overlap were too great to effectively exclude any arthropods from the plants. Experiments were conducted on two *Clitoria racemosa* and one *Coffea arabica.* I conducted ant exclusion experiments over 16 weeks, and arthropod and predator exclusion experiments over six weeks. Experiments began on one individual of each species that had high infestations of mealybugs, allowing for several replicates within each plant. After six weeks, an infestation on another *Clitoria racemosa* was observed, and I included it in the experiment.

Experiments involved the four treatments listed in Table 1. In all treatments, leaves were selected where mealybugs were already present, as the goal of the exclusion experiments was to track changes in mealybug numbers. Treatment A-P+ involved applying Tanglefoot resin to the stem immediately distal to the experimental leaf, excluding ants from the leaf. In treatment A-P-, a net covered the leaf and I applied Tanglefoot to the base of the stem, to exclude all arthropods except mealybugs already present from accessing that leaf. Treatment A+P+ allowed the presence of any arthropod in the rainforest biome. Treatment A+P+ consisted of covering the leaf with a net to exclude all arthropods, except for ants, from the leaf.

These exclusion experiments relied on several assumptions. The first assumption is that putative predators of the mealybugs, such as spiders, were more likely to drop onto leaves than to walk up stems. Therefore, Tanglefoot at the base of the leaf did not exclude them from that leaf, but covering the leaf surface with a net did. The second assumption is that ants accessed the leaf by crawling up the stem rather than by dropping onto the surface of leaves. Tanglefoot at the base of a leaf effectively excluded the ants from that leaf, but a net covering the surface of the leaf did not. Third, due to the size difference between putative predators and ants, nets that covered the leaf and were tied at the base of the stem were assumed to be effective at excluding predators but not ants from the leaf. These assumptions were based on observational evidence in pilot experiments, such as finding spiders on leaves where Tanglefoot had been applied to the stem but not finding ants on them. Observational evidence also included *P. longicornis* found inside nets that covered leaves and had been tied at the base of the stem, but not finding spiders inside them.

Ant Exclusion

I began with Treatments A-P+ and A+P+, to test only whether, in the presence of predators, the presence or absence of ants affected mealybug numbers. Other arthropods were not excluded from these treatments. The experiment consisted of a total of 12 replicates on one *Clitoria racemosa* individual (six A-P+ and six A+P+), eight replicates on the *Coffea arabica* individual (four A-P+ and four A+P+), and four replicates on the second *Clitoria racemosa* (two A-P+ and two A+P+). The same procedure was followed for setting up each replicate. Two leaves that had mealybugs present were first tagged. The ant exclusion treatment, A-P+, entailed applying Tanglefoot to the stem immediately distal to the experimental leaf, which prevented ants from accessing that leaf. To ensure that ants were not able to access experimental leaves from another entry point, I only selected experimental leaves that did not touch other leaves. Any ants present on the leaf were removed with forceps before I applied Tanglefoot. The second leaf was unmanipulated, allowing free access to all arthropods, including ants. Every week I returned to reapply Tanglefoot to the experimental leaves when necessary, and to take photos of each leaf. An assistant uploaded these images to ImageJ and recorded the number of mealybugs present on each leaf using the Cell Counter plugin. The number of experiments replicated between trees eventually varied: three weeks into the experiment, a *M. acuminata* fell into one of the *Clitoria racemosa* individuals, causing four of the six exclusion replicates on that individual to be compromised, as ants were no longer excluded from the experimental leaves.

All-Arthropod Exclusion and Predator Exclusion

I added treatments A-P- and A+P- (Table 1) several weeks into the experiment, as mealybug numbers were initially low, or colonies were located high in the canopy and not accessible on foot. A-P- and A+P- were added later because they were more difficult not as simple to set up as A+P+ and A-P+. A-P- and A+P- required mealybug colonies to be on leaves that could be easily covered with nets and accessed on foot, and colonies like this did not appear until several weeks into the experiment. I tested the effects on mealybug numbers of excluding ants as well as all other arthropods, including potential mealybug predators, over a 6-week period. Putative predators included arachnid species, which were found in species surveys in the rainforest biome (see Chapter 1) and which were seen at times on the leaves in the ant exclusion experiments. As mentioned above, it was possible for predators to gain access to leaves in the ant exclusion experiments, as they were able to create webbing to move from leaf to leaf. The experiment consisted of three replicates, one a *Clitoria racemosa* and one on a *Coffea arabica* individual, and an additional replicate on a second *Clitoria racemosa* tree (only one replicate was done on this tree, due to low mealybug numbers). The same procedure was followed for each replicate. Treatments again started with labeling two leaves on which mealybug colonies were present. To set up treatment A-P-, Tanglefoot was applied to the stem immediately distal to the experimental leaf, as in the ant exclusion experiment, but in addition, the leaf was covered with a mesh net. The mesh allowed only for the passage of air, and blocked predators from moving onto the surface of leaves, as they had been able to do in Treatment A-P+. Treatment A+P-, which looked at mealybug numbers when only ants were given access to leaves, involved covering a leaf with a net and tying the net at the base of the stem. The net again blocked predators from moving onto the surface of leaves, but ants were still able to access the leaf from the stem. I followed the same procedure as in the A+P+, A-P+ treatments to record mealybug numbers, returning every week to take pictures, and passing them to an assistant who recorded the numbers with the ImageJ Cell Counter plugin.

Statistical Analyses

Analyses and significance tests were performed using R 3.4.1 (R Core Team 2017). To determine the difference in mealybug numbers between specific pairs of treatments, a series of four generalized linear mixed models (GLMM) were employed in the lmm package (Schafer 2018) on the dependent variable, difference in mealybug number. Initial mealybug numbers were a covariate. Residuals were checked using the h.res function in the car package (Fox et al. 2018). These models tested for the effects the initial number of mealybugs and treatment type had on the difference seen in mealybug numbers. For each treatment comparison GLMM, the initial number of mealybugs was a fixed effect while the start date was a random effect. Significance values were calculated using Wald χ^2 *tests in the car package (Fox et al. 2018), and used type II sum of squares (Langsrud 2003).*

Results

Treatment A+P+ showed no significant change in the difference in mealybug numbers compared to treatment A+P- (Figure 1a, GLMM for treatment effect, P =0.363), contrary to my prediction that mealybug numbers in A+P- should increase when compared to A+P+ (Table 1). No significant difference between these treatments suggests that predator numbers are not having a strong enough effect on mealybugs for there to be a significant difference in population size when they are excluded.

Treatment A-P+ showed no significant change in the difference in mealybug numbers compared to treatment A-P- (Figure 1b, GLMM for treatment effect P =0.335), also contrary to my prediction that mealybug numbers in A-P- should increase compared to A-P+ (Table 1). No significant difference between these treatments suggests that mealybugs do not require ant protection, as predators do not have a significant effect on mealybug numbers when they are allowed access to them, and ants are excluded.

However, treatment A+P+ showed a significant difference in mealybug numbers compared to treatment A-P+ (Figure 1c, GLMM for treatment effect P =0.049). The average numbers of mealybugs removed from treatments A+P+ and A-P+ over the course of the experiment were then calculated to determine if the predicted outcome of A-P+ decreasing in mealybug number compared to A+P+ was correct. The average magnitude of decrease in mealybugs from treatment A-P+ was found to be 45.4 mealybugs (SD= 26.9 mealybugs) whereas the average number of mealybugs removed from A+P+ was found to be 19.9 mealybugs (s = 37.75 mealybugs). Thus, more mealybugs were lost in treatment A-P+ compared to A+P+, which is the predicted outcome if ants are reducing the effects predators are having on mealybugs, i.e., if there is a mutualism.

Finally, treatment A-P- showed no significant change in the difference in mealybug numbers compared to treatment A+P- (Figure 1d, GLMM for treatment effect P =0.168), as predicted in Table 1. This result suggests ants are not harming mealybug populations, as there is no change in mealybug numbers when predators and ants are entirely excluded, and predators are excluded but ants are allowed access.

Figure 1: Number of Mealybugs Lost Per Treatment

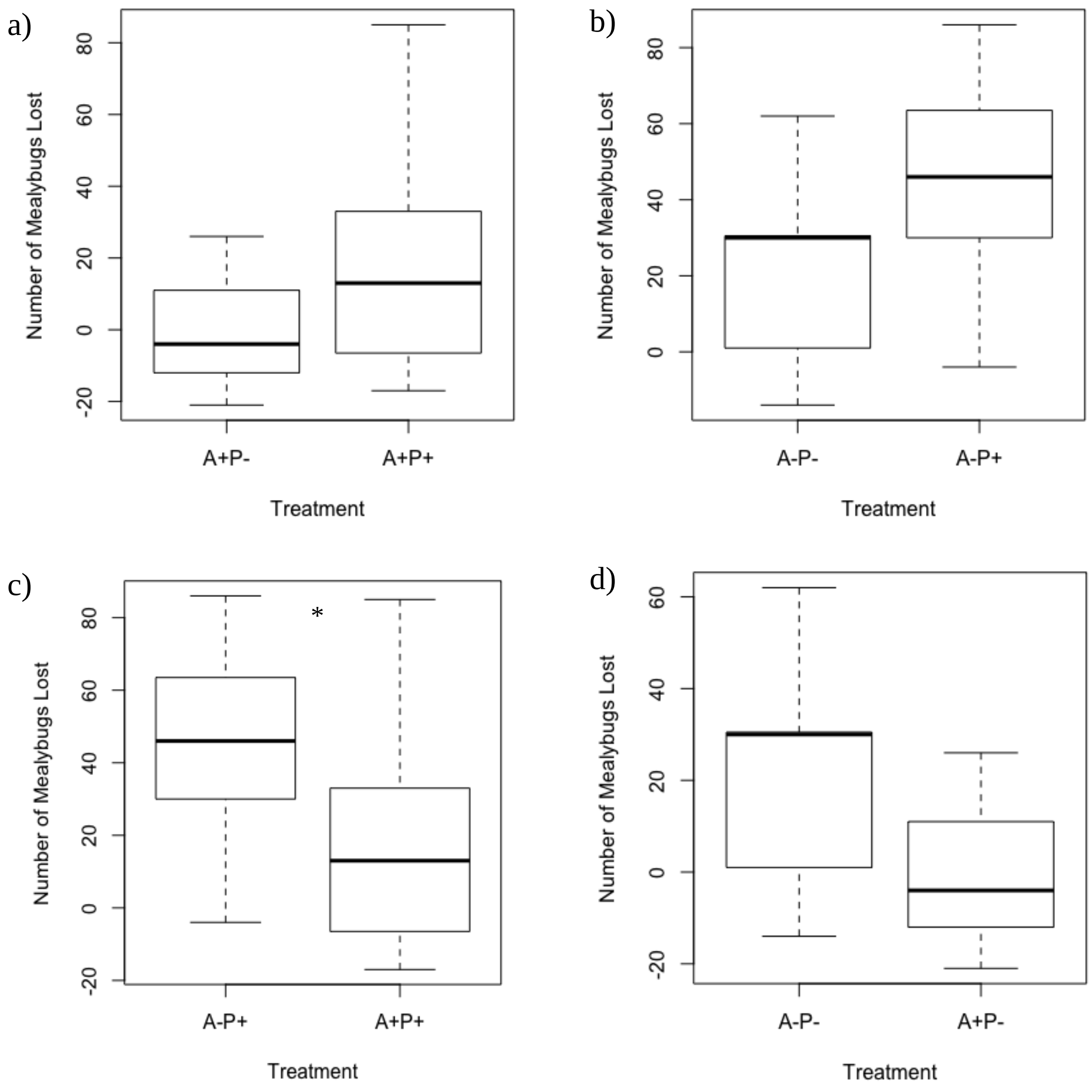

Figure 1: Effect of treatment on number of mealybugs lost when compared between specific treatments. Bolded line represents the estimated mean and error bars represent estimated mean and SEM. The only significant difference in mealybug numbers is seen in Figure 1c between treatments A-P+ and A+P+ and is indicated as significant at the 0.05 level with a symbol *.

Table 2: Paired Treatment Comparison Results

Treatments Compared	Change in mealybugs between treatments observed?	P-value
A+P+ v. A+P-	No	0.363
A-P+ v. A-P-	No	0.335
A+P+ v. A-P+	Yes	0.049*
A-P- v. A+P-	No	0.168

Table 2: P-values from chi-squared tests run for specific treatment pairings.

The average mealybug numbers in A+P+, the unmanipulated control treatment, vary from week to week over a 16-week period (seen in Figure 2). Mealybug numbers were calculated as the average number of mealybugs for each A+P+ replicate. This figure shows that mealybug population numbers are variable even without treatments that allow or deny different species access to their colonies, and suggesting other environmental or life history factors are causing variation in mealybug numbers.

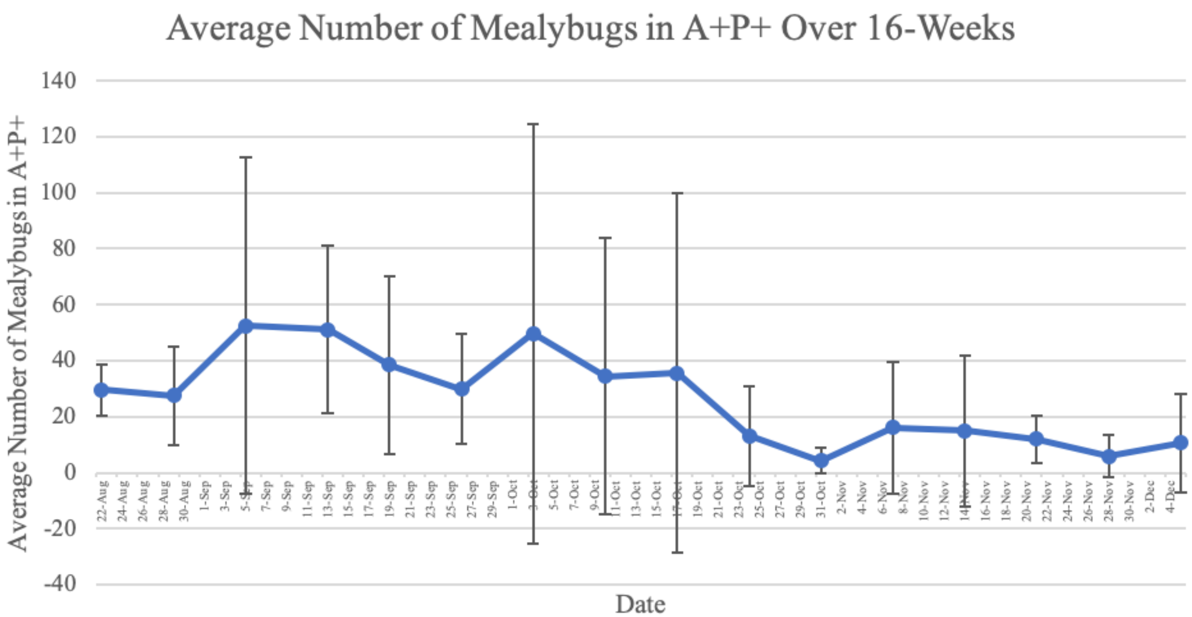

Figure 2: Average number of mealybugs in unmanipulated, control treatment A+P+ over a 16-week period.

Discussion

The evidence for a mutualism between ants and mealybugs within the rainforest biome of Biosphere 2, is inconsistent. If this interaction is occurring, the effect is extremely weak, possibly due to the rarity of predators. Contrary to predictions, there were no significant differences in mealybug numbers when A+P+ and A+P- were compared and when A-P+ and A-P- were compared (Table 2).The former comparison implies that predators are not having an effect on mealybug populations at this site, as there is no difference in mealybug numbers when they are removed compared to when they are present. This could be because predators are not at high abundance within Biosphere 2. The only putative predator of mealybugs found in the 2018 species surveys were spiders (Chapter 1), and they were recorded in low numbers relative to other species within Biosphere 2, such as ants. Therefore, predator numbers could be too low to have a serious effect on mealybug populations. Additionally, no significant change was seen in mealybug numbers in treatments A-P+ and A-P-, again strongly indicating that a mutualism is not occurring: this result suggests that mealybugs do not require protection at this site. Predators are either not preying on mealybugs, or predators are at abundances too low to impact mealybug populations.

Contrary to this evidence for a lack of mutualism, however, there was a weakly significant difference in the change in mealybug numbers between treatments A+P+ and A-P+ (Table 2). This result suggests that ants are having a weak beneficial effect for mealybug numbers. It is possible they are deterring any predators that may be present. However, as predators are at low abundances in the system and their presence or absence does not seem to affect mealybug numbers (Figure 1), the presence of ants could be benefiting mealybug colonies in another way. For example, ants have been known to provide other benefits to mealybugs beyond protection, such as preventing the build-up of honeydew, therefore reducing mortality risks from fungal attacks (Buckley 1987*a*).

Mealybug numbers are fluctuating in Biosphere 2. Even when unmanipulated, mealybug populations in Biosphere 2 are highly variable. The average mealybug numbers in treatment A+P+ over a 16-week period (seen in Figure 2) consistently vary from week to week. Mealybug numbers can change in nature due to a wide variety of abiotic and biotic factors, including temperature, season, host plant type, host plant quality, and reproductive traits (Chong & Mannion 2008, Aheer et al. 2009, Nisha & Kennedy 2017, Sadof et al. 2003). These are all factors that could be causing fluctuations in the Biosphere 2. In the future, the seasonal abundance of the mealybug species could be examined. Observations made by Biosphere 2 employees, including John Adams, Deputy Director of Biosphere 2, indicate that mealybug numbers rise and fall depending on the time of year, rising as the temperature gets warmer. These observations suggest that seasonality could be a factor affecting the number of mealybugs.

The uncertain results of this study offer the opportunity for further research to be carried out in Biosphere 2. For instance, tracking and establishing baseline mealybug numbers in this system over a longer period of time, could aid in understanding the fluctuations in their numbers.

Another possibility for future research in this system could be releasing natural predators into the system and determining if a stronger mutualism between the ants and the mealybugs emerges. Ant and predator exclusion treatments similar to the ones done in this experiment could be carried out, and the effect on mealybug numbers could be tracked. If no mutualism is observed when there are higher predator numbers, it could shed insight into how interactions change from expected outcomes, in highly-disturbed systems such as Biosphere 2, where a novel biotic community is assembled.

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